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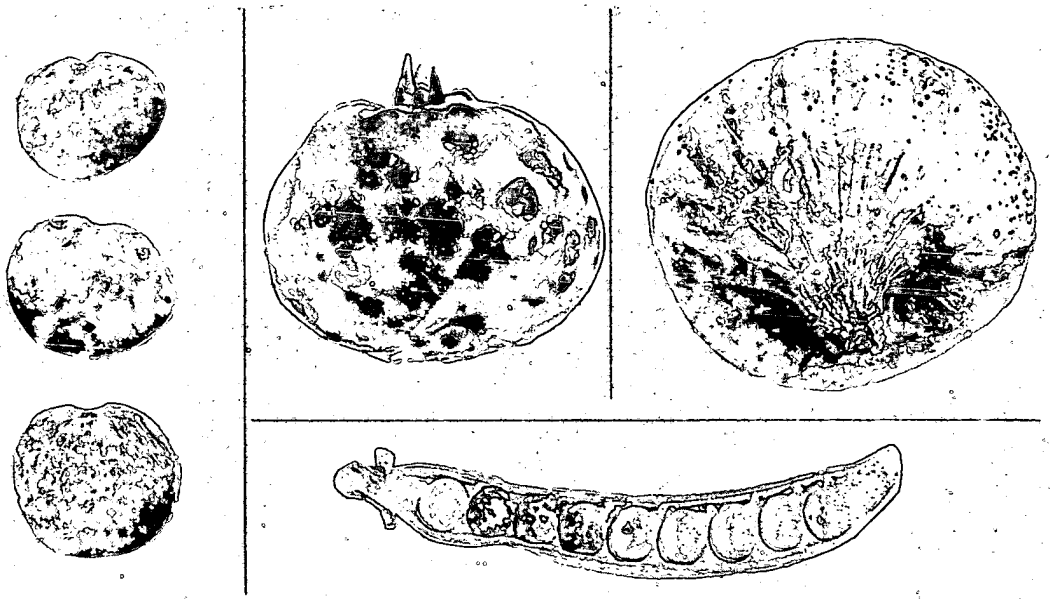

Modern Food Microbiology

THIRD EDITION

JAMES M. JAY

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VAN NOSTRAND REINHOLD
AVI

11.

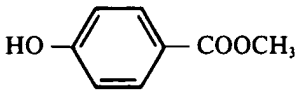
FOOD PRESERVATION WITH CHEMICALS

The use of chemicals to prevent or delay the spoilage of foods derives in part from the fact that such compounds have been used with great success in the treatment of diseases of man, animals, and plants. This is not to imply that any and all chemotherapeutic compounds can or should be used as food preservatives. On the other hand, there are some chemicals of value as food preservatives that would be ineffective or too toxic as chemotherapeutic compounds. With the exception of certain antibiotics, none of the presently used food preservatives find any real use as chemotherapeutic compounds in man and animals. While a large number of chemicals have been described that show potential as food preservatives, only a relatively small number are allowed in food products. This is due in large part to the strict rules of safety adhered to by the Food and Drug Administration (FDA), and to a lesser extent to the fact that not all compounds that show antimicrobial activity in vitro do so when added to certain foods. Below are described those compounds most widely used, their modes of action where known, and the types of foods in which they are used. Those chemical preservatives generally recognized as safe (GRAS) are summarized in Table 11-1.

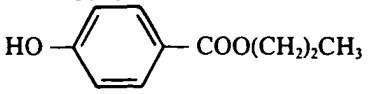
BENZOIC ACID AND THE PARABENS

Benzoic acid (C_6H_5COOH) and its sodium salt ($C_7H_5NaO_2$) along with the esters of *p*-hydroxybenzoic acid (parabens) are considered together in this section. Sodium benzoate was the first chemical preservative permitted in foods by the U.S. Food and Drug Administration, and it continues in wide use today in a large number of foods. Its approved derivatives have structural formulas as noted:

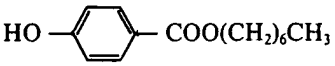
Methylparaben
Methyl *p*-Hydroxybenzoate



Propylparaben
Propyl *p*-Hydroxybenzoate



Heptylparaben
n-Heptyl-*p*-hydroxybenzoate.



The antimicrobial activity of benzoate is related to pH, the greatest activity being at low pH values. The antimicrobial activity resides in the undissociated molecule (see below). These compounds are most active at the lowest pH values of foods and essentially ineffective at neutral values. The pK of benzoate is 4.20 and at a pH of 4.00, 60% of the compound is undissociated, while at a pH of 6.0 only 1.5% is undissociated. This results in the restriction of benzoic acid and its sodium salts to high-acid products such as apple cider, soft drinks, tomato catsup, and salad dressings. High acidity alone is generally sufficient to prevent growth of bacteria in these foods, but not that of certain molds and yeasts. As used in acidic foods, benzoate acts essentially as a mold and yeast inhibitor although it is effective against some bacteria in the 50–500 ppm range. Against yeasts and molds at around pH 5.0–6.0, from 100–500 ppm are effective in inhibiting the former, while for the latter from 30–300 ppm are inhibitory.

In foods such as fruit juices, benzoates may impart disagreeable tastes at the maximum level of 0.1%. The taste has been described as being "peppery" or burning.

As noted above, the three parabens that are permissible in foods in the United States are heptyl-, methyl-, and propyl-, while butyl- and ethylparabens are permitted in food in certain other countries. As esters of *p*-hydroxybenzoic acid, they differ from benzoate in their antimicrobial activity in being less sensitive to pH. Although not as many data have been presented on heptylparaben, it appears to be quite effective against microorganisms, with 10–100 ppm effecting complete inhibition of some gram-positive and gram-negative bacteria. Propylparaben is more effective than methylparaben on a ppm basis, with up to 1,000 ppm of the former and 1,000–4,000 ppm of the latter needed for bacterial inhibition, with gram-positive bacteria being more susceptible than gram negatives to the parabens in general (20). Heptylparaben has been reported to be effective against the malo-lactic bacteria. In a reduced-broth medium, 100 ppm propylparaben delayed germination and toxin production by *C. botulinum* type A, while 200 ppm effected inhibition up to 120 h at 37°C (100). In the case of methylparaben, 1,200 ppm were required for inhibition similar to that for the propyl- analog.

The parabens appear to be more effective against molds than against yeasts. As in the case of bacteria, the propyl- derivative appears to be the most effective where 100 ppm or less are capable of inhibiting some yeasts

Table 11-1. Summary of some GRAS chemical food preservatives.

Preservatives	*Maximum tolerance*	*Organisms affected*	*Foods*
Propionic acid/ propionates	0.32%	Molds	Bread, cakes, some cheeses, rope inhibitor in bread dough
Sorbic acid/ sorbates	0.2%	Molds	Hard cheeses, figs, syrups, salad dressings, jellies, cakes
Benzoic acid/ benzoates	0.1%	Yeasts and molds	Margarine, pickle relishes, apple cider, soft drinks, tomato catsup, salad dressings
Parabens[a]	0.1%[b]	Yeasts and molds	Bakery products, soft drinks, pickles, salad dressings
SO_2/sulfites	200–300 ppm	Insects, micro-organisms	Molasses, dried fruits, wine making, lemon juice (not to be used in meats or other foods recognized as sources of thiamine)
Ethylene/ propylene oxides[c]	700 ppm	Yeasts, molds, vermin	Fumigant for spices, nuts
Sodium diacetate	0.32%	Molds	Bread
Dehydroacetic acid	65 ppm	Insects	Pesticide on strawberries, squash
Sodium nitrite[c]	120 ppm	Clostridia	Meat-curing preparations
Caprylic acid	—	Molds	Cheese wraps
Ethyl formate	15–200 ppm[d]	Yeasts and molds	Dried fruits, nuts

GRAS (Generally Recognized As Safe) per Section 201 (32)(s) of the U.S. Federal Food, Drug, and Cosmetic Act as amended.
[a] Methyl-, propyl-, and heptyl-esters of *p*-hydroxybenzoic acid.
[b] Heptyl ester—12 ppm in beers; 20 ppm in noncarbonated and fruit-based beverages.
[c] May be involved in mutagenesis and/or carcinogenesis.
[d] As formic acid.

and molds, while for heptyl- and methylparabens, 50–200 and 500–1,000 ppm, respectively, are required.

Like benzoic acid and its sodium salt, the methyl- and propylparabens are permissible in foods up to 0.1% while heptylparaben is permitted in beers to a maximum of 12 ppm, and up to 20 ppm in fruit drinks and beverages. The pK for these compounds is around 8.47, and their antimicrobial activity is not increased to the same degree as for benzoate with the lowering of pH as noted above. They have been reported to be effective at pH values up to 8.0. For a more thorough review of these preservatives, see Davidson (20).

Similarities between the modes of action of benzoic and salicylic acids have been noted (8). Both compounds, when taken up by respiring microbial cells, were found to block the oxidation of glucose and pyruvate at the acetate level in *Proteus vulgaris*. With *P. vulgaris*, benzoic acid caused an increase in the rate of O_2 consumption during the first part of glucose oxidation (8). The benzoates, like propionate and sorbate, have been shown to act against microorganisms by inhibiting the cellular uptake of substrate molecules (36). The stage of endospore germination most sensitive to benzoate is noted in Fig. 11-1.

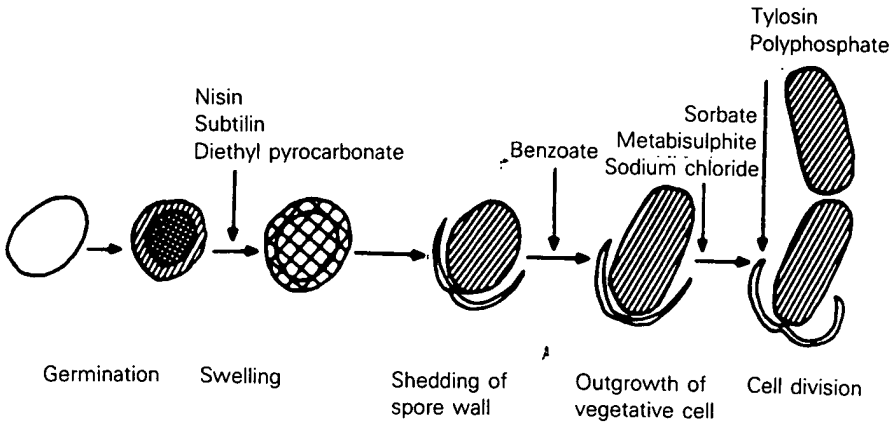


Fig. 11-1. Diagrammatic representation of growth of an endospore into vegetative cells showing stages arrested by minimum inhibitory concentrations of some food preservatives (40).

The undissociated form is essential to the antimicrobial activity of benzoate as well as for other lipophilic acids such as sorbate and propionate, as previously noted. In this state, these compounds are soluble in the cell membrane and act apparently as proton ionophores (41). As such, they facilitate proton leakage into cells and thereby increase energy output of cells to maintain their usual internal pH. With the disruption in membrane activity, amino acid transport is adversely affected (41).

SORBIC ACID

Sorbic acid ($CH_3CH{=}CHCH{=}CHCOOH$) is employed as a food preservative usually as the calcium, sodium, or potassium salt. These compounds are permissible in foods at levels not to exceed 0.2%. Like sodium benzoate, they are more effective in acid foods than in neutral foods and tend to be on par with the benzoates as fungal inhibitors. Sorbic acid works best below pH 6.0 and is generally ineffective > pH 6.5. These compounds are more effective than sodium benzoate between pH 4.0–6.0. At pH values of 3.0 and below, the sorbates are slightly more effective than the propionates

but about the same as sodium benzoate. The pK of sorbate is 4.80 and at a pH of 4.0, 86% of the compound is undissociated while at a pH of 6.0 only 6% is undissociated. Sorbic acid can be employed in cakes at higher levels than propionates without imparting flavor to the product (79).

The sorbates are primarily effective against molds and yeasts but research during the past decade has shown them to be effective against a wide range of bacteria. In general, the catalase-positive cocci are more sensitive than the catalase negatives, and aerobes are more sensitive than anaerobes. The resistance of the lactic acid bacteria to sorbate, especially at pH 4.5 or above, permits its use as a fungistat in products that undergo lactic fermentations. Its effectiveness has been shown against *S. aureus,* salmonellae, coliforms, psychrotrophic spoilage bacteria (especially the pseudomonads), and *V. parahaemolyticus*. Against the latter organism, concentrations as low as 30 ppm have been shown to be effective. Shelf-life extensions have been obtained by use of sorbates on fresh poultry meat, vacuum-packaged poultry products, fresh fish, and perishable fruits. For further information, see nitrite-sorbate combinations below, and the review by Sofos and Busta (112).

The sorbates have been studied by a large number of groups for use in meat products in combination with nitrites. Bacon formulations that contain 120 ppm $NaNO_2$ without sorbate yield products that maintain their desirable organoleptic qualities in addition to being protected from *C. botulinum* growth. When 0.26% (2,600 ppm) potassium sorbate is added along with 40 ppm nitrite, no significant differences are found in the organoleptic qualities or in botulinal protection ([57] and Stevenson and Price, 1976, cited in [87]). The combination of 40 ppm $NaNO_2$ and 0.26% potassium sorbate (along with 550 ppm sodium ascorbate or sodium erythrobate) was proposed by the U.S. Department of Agriculture (USDA) in 1978 but postponed in 1979. The later action was prompted not by the failure of the reduced nitrite level in combination with sorbate but because of taste panel results that characterized finished bacon as having "chemical"-like flavors and producing prickly mouth sensations (3). The combination of sorbate plus reduced nitrite has been shown to be effective in a variety of cured meat products against not only *C. botulinum* but other bacteria such as *S. aureus*. (For further information, see nitrite section below, and reviews by Sofos and Busta [112], Tompkin [123], and Liewen and Marth [73].)

The widest use of sorbates is as fungistats in products such as cheeses, bakery products, fruit juices, beverages, salad dressings, and the like. With molds, inhibition has been reported to be due to inhibition of the dehydrogenase enzyme system (24), and to the inhibition of cellular uptake of substrate molecules such as amino acids (see benzoic acid section above), phosphate, organic acids, and so on (36). A number of other possible inhibitory mechanisms have been presented by various researchers. Against germinating endospores, sorbate prevents the outgrough of vegetative cells (Fig. 11-1).

With respect to toxicity, sorbic acid is metabolized in the body to CO_2 and H_2O in the same manner as fatty acids normally found in foods (26).

THE PROPIONATES

Propionic acid is a three-carbon organic acid with the following structure: CH_3CH_2COOH. This acid and its calcium and sodium salts are permitted in breads, cakes, certain cheeses, and other foods primarily as a mold inhibitor. Propionic acid is employed also as a "rope" inhibitor in bread dough. The tendency toward dissociation is low with this compound and its salts, and these compounds are consequently active in low-acid foods. They tend to be highly specific against molds, with the inhibitory action being primarily fungistatic rather than fungicidal.

With respect to the antimicrobial mode of action of propionates, they act in a manner similar to that of benzoate and sorbate. The pK of propionate is 4.87 and at a pH of 4.00, 88% of the compound is undissociated, while at a pH of 6.0, only 6.7% remains undissociated. The undissociated molecule of this lipophilic acid is necessary for its antimicrobial activity. The mode of action of propionic acid is noted above with benzoic acid. See also section below on medium-chain fatty acids and esters, and review by Doores (28) for further information.

SULFUR DIOXIDE AND SULFITES

Sulfur dioxide (SO_2) and the sodium and potassium salts of sulfite ($=SO_3$), bisulfite ($—HSO_3$), and metabisulfite ($=S_2O_5$) all appear to act similarly and are here treated together. Sulfur dioxide is used in its gaseous or liquid form, or in the form of one or more of its neutral or acid salts on dried fruits, in lemon juice, molasses, wines, fruit juices, and others. The parent compound has been used as a food preservative since ancient times. Its use as a meat preservative in the United States dates back to at least 1813; however, it is not permitted in meats or other foods recognizable as sources of thiamine. While SO_2 possesses antimicrobial activity, it is also used in certain foods as an antioxidant.

The predominant ionic species of sulfurous acid depends upon pH of milieu with SO_2 being favored by pH < 3.0, HSO_3^- by pH between 3.0 and 5.0, and $SO_3^=$ > pH 6.0 (86). SO_2 has pKs of 1.76 and 7.2. The sulfites react with various food constituents including nucleotides, sugars, disulfide bonds, and others.

With regard to its effect on microorganisms, SO_2 is bacteriostatic against *Acetobacter* spp. and the lactic acid bacteria at low pH, concentrations of 100 to 200 ppm being effective in fruit juices and beverages. It is bactericidal at higher concentrations. When added to temperature-abused comminuted pork, 100 ppm of SO_2 or higher were required to effect significant inhibition of spores of *C. botulinum* at target levels of 100 spores/g (127). The source

of SO_2 was sodium metabisulfite. Employing the same salt to achieve an SO_2 concentration of 600 ppm, Banks and Board (2) found that growth of salmonellae and other Enterobacteriaceae were inhibited in British fresh sausage. The most sensitive bacteria were eight salmonellae serovars, which were inhibited by 15–109 ppm at pH 7.0, while *Serratia liquefaciens, S. marcescens,* and *Hafnia alvei* were the most resistant, requiring 185–270 ppm free SO_2 in broth.

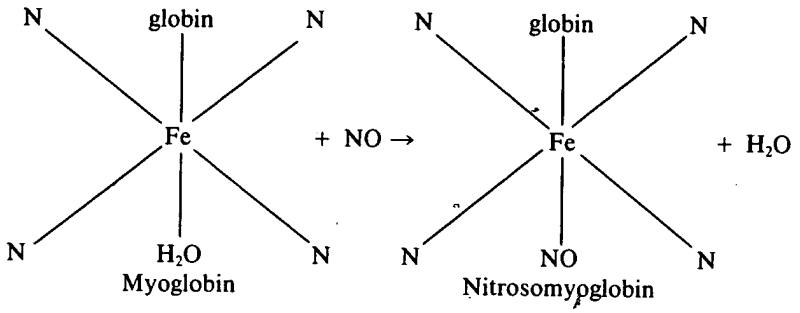
Yeasts are intermediate to acetic and lactic acid bacteria and molds in their sensitivity to SO_2, and the more strongly aerobic species are generally more sensitive than the more fermentative species (64). Sulfurous acid at levels of 0.2–20 ppm was effective against some yeasts, including *Saccharomyces, Pichia,* and *Candida,* while *Zygosaccharomyces bailii* required up to 230 ppm for inhibition in certain fruit drinks at pH 3.1 (76). Yeasts can actually form SO_2 during juice fermentation—some *S. carlsbergensis* and *S. bayanus* strains produce up to 1,000 and 500 ppm, respectively (86). Molds such as *Botrytis* can be controlled on grapes by periodic gassing with SO_2, and bisulfite can be used to destroy aflatoxins (29). Both aflatoxins B_1 and B_2 can be reduced in corn (44, 82). Sodium bisulfite was found to be comparable to propionic acid in its antimicrobial activity in corn containing up to 40% moisture (44).

Although the actual mechanism of action of SO_2 is not known, several possibilities have been suggested, each supported by some experimental evidence. One suggestion is that the undissociated sulfurous acid or molecular SO_2 is responsible for the antimicrobial activity. Its greater effectiveness at low pH tends to support this. Vas and Ingram (129) suggested the lowering of pH of certain foods by addition of acid as a means of obtaining greater preservation with SO_2. It has been suggested that the antimicrobial action is due to the strong reducing power that allows these compounds to reduce oxygen tension to a point below that at which aerobic organisms can grow, or by direct action upon some enzyme system. SO_2 is also thought to be an enzyme poison, inhibiting growth of microorganisms by inhibiting essential enzymes. Its use in the drying of foods to inhibit enzymatic browning is based upon this assumption. Since the sulfites are known to act on disulfide bonds, it may be presumed that certain essential enzymes are affected and that inhibition ensues. The sulfites do not inhibit cellular transport. It may be noted from Fig. 11-1 that metabisulfite acts on germinating endospores during the outgrowth of vegetative cells.

NITRITES AND NITRATES

Sodium nitrate ($NaNO_3$) and sodium nitrite ($NaNO_2$) are used in curing formulae for meats since they stabilize red meat color, inhibit some spoilage and food poisoning organisms, and contribute to flavor development. The role of NO_2 in cured meat flavor has been reviewed by Gray and Pearson (43). NO_2 has been shown to disappear both on heating and storage. It

should be recalled that many bacteria are capable of utilizing nitrate as an electron acceptor and in the process effect its reduction to nitrite. The nitrite ion is by far the more important of the two in preserved meats. This ion is highly reactive and is capable of serving both as a reducing and an oxidizing agent. In an acid environment, it ionizes to yield nitrous acid (3HONO). The latter further decomposes to yield nitric oxide (NO), which is the important product from the standpoint of color fixation in cured meats. Ascorbate or erythrobate acts also to reduce NO_2 to NO. Nitric oxide reacts with myoglobin under reducing conditions to produce the desirable red pigment **nitrosomyoglobin,** as shown in the following (see also Table 9-9):



When the meat pigment exists in the form of **oxymyoglobin,** as would be the case for comminuted meats, this compound is first oxidized to **metmyoglobin** (brown color). Upon the reduction of the latter, nitric oxide reacts to yield nitrosomyoglobin. Since nitric oxide is known to be capable of reacting with other porphyrin-containing compounds such as catalase, peroxidases, cytochromes, and others, it is conceivable that some of the antibacterial effects of nitrites against aerobes may be due to this action (the mechanism is discussed below). It has been shown that the antibacterial effect of NO_2 increases as pH is lowered within the acid range, and this effect is accompanied by an overall increase in the undissociated HNO_2 (13).

Organisms affected

Although the single microorganism of greatest concern relative to nitrite inhibition is *C. botulinum,* the compound has been evaluated as an antimicrobial for other organisms. During the late 1940s it was evaluated as a fish preservative and found to be somewhat effective but generally only at low pH. It is effective against *S. aureus* at high concentrations and, again, the effectiveness increases as pH is lowered. The compound is generally ineffective against Enterobacteriaceae, including the salmonellae, and against the lactic acid bacteria, although some effects are noted in cured and in vacuum-packaged meats and are probably caused by the interaction of nitrite with other environmental parameters rather than to nitrite alone.

Nitrite is added to cheeses in some countries to control gassiness caused by *Clostridium butyricum* and *C. tyrobutyricum*. It is effective against other clostridia including *C. sporogenes* and *C. perfringens*, which are often employed in laboratory studies to assess potential antibotulinal effects not only of nitrites but of other inhibitors that might have value as nitrite adjuncts or sparing agents.

The Perigo factor

The almost total absence of botulism in cured, canned, and vacuum-packed meats and fish products led some investigators in the mid-1960s to seek reasons as to why meat products that contained viable endospores did not become toxic. Employing culture medium, it was shown in 1967 that about ten times more nitrite was needed to inhibit clostridia if it were added after instead of before the medium was autoclaved. It was concluded that the heating of the medium with nitrite produced a substance or agent about ten times more inhibitory than nitrite alone (89, 90). This agent is referred to as the **Perigo factor.** The existence of this factor or effect has been confirmed by some and questioned by others. While the Perigo factor may be questionable in cured and perishable cured meats, the evidence for an inhibitory factor in culture media involving nitrite, iron, and —SH groups is more conclusive (123).

The factor does not develop in all culture media, and heating to at least 100°C is necessary for its development, although some activity develops in meats when heated to as low as 70°C. The Perigo factor is dialyzable from some culture media and meat suspensions but not from other media (62). It is not found in filter-sterilized solutions of the same medium with nitrite added (104). It has been shown that if meat is added to a medium containing the Perigo factor, the inhibitory activity is lost (63). For this reason, some Canadian workers call the inhibitor that is formed in meat the "Perigo-type factor" (14).

It is this inhibitory or antibotulinal effect that results from the heat processing or smoking of certain meat and fish products containing nitrite that warrants the continued use of nitrite in such products. The antibotulinal activity of nitrite in cured meats is of greater public health importance than the facts of color and flavor development. For the latter, initial nitrite levels as low as 15 to 50 ppm have been reported to be adequate for various meat products including Thuringer sausage (25). Nitrite levels of 100 ppm or more have been found to make for maximum flavor and appearance in fermented sausages (70). The antibotulinal effect requires at least 120 ppm for bacon (9, 17), comminuted cured ham (16), and canned, shelf-stable luncheon meat (14). Many of these canned products are given a low heat process (F_o of 0.1–0.6).

Interaction with cure ingredients and other factors

The interplay of all ingredients and factors involved in heat-processed, cured meats on antibotulinal activity was noted over 20 years ago by Riemann

(98), and several other investigators have pointed out that curing salts in semipreserved meats are more effective in inhibiting heat-injured spores than noninjured (31, 103). With brine and pH alone, higher concentrations of the former are required for inhibition as pH increases, and Chang et al. (14) suggested that the inhibitory effect of salt in shelf-stable canned meats against heat-injured spores may be more important than the Perigo-type factor. With smoked salmon inoculated with 10^2 spores/g of *C. botulinum* types A and E and stored in O_2-impermeable film, 3.8 and 6.1% water-phase NaCl alone inhibited toxin production in 7 days by types E and A, respectively (87). With 100 ppm or more of NO_2, only 2.5% NaCl was required for inhibition of toxin production by type E, and for type A 3.5% NaCl + 150 ppm $NaNO_2$ was inhibitory. With longer incubations or larger spore inocula, more NaCl or $NaNO_2$ is needed.

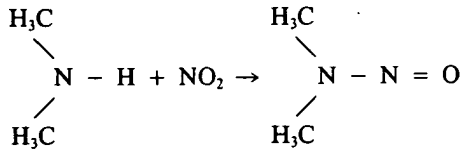
The interplay of NaCl, $NaNO_2$, $NaNO_3$, isoascorbate, polyphosphate, thermal process temperatures, and temperature/time of storage on spore outgrowth and germination in pork slurries has been studied extensively by Roberts et al. (101), who found that significant reductions in toxin production could be achieved by increasing the individual factors noted. It is well known that low pH is antagonistic to growth and toxin production by *C. botulinum,* whether the acidity results from added acids or the growth of lactic acid bacteria. When 0.9% sucrose was added to bacon along with *Lactobacillus plantarum,* only one of forty-nine samples became toxic after 4 weeks, while with sucrose and no lactobacilli, fifty of fifty-two samples became toxic in 2 weeks (119). When 40 ppm nitrite was used alone, forty-seven of fifty samples became toxic after 2 weeks but when 40 ppm nitrite was accompanied by 0.9% sucrose and an inoculum of *L. plantarum,* none of thirty became toxic. While this was most likely a direct pH effect, other factors may have been involved (see section on lactic antagonism in Chapter 16). In more recent studies, bacon was prepared with 40 or 80 ppm $NaNO_2$ + 0.7% sucrose followed by inoculation with *Pediococcus acidilactici.* When inoculated with *C. botulinum* types A and B spores, vacuum-packaged, and incubated up to 56 days at 27°C, the bacon was found to have greater antibotulinal properties than control bacon prepared with 120 ppm $NaNO_2$ but not sucrose or lactic inoculum (118). Bacon prepared by the above formulation, called the Wisconsin process, was preferred by a sensory panel to that prepared by the conventional method (117). The Wisconsin process employs 550 ppm of sodium ascorbate or sodium erythrobate, as does the conventional process.

Nitrosamines

When nitrite reacts with secondary amines, **nitrosamines** are formed, and many are known to be carcinogenic. The generalized way in which nitrosamines may form is as follows:

$$R_2NH_2 + HONO \xrightarrow{H^+} R_2N{-}NO + H_2O$$

The amine dimethylamine reacts with nitrite to form N-nitrosodimethylamine:



In addition to secondary amines, tertiary amines and quarternary ammonium compounds also yield nitrosamines with nitrite under acidic conditions. Nitrosamines have been found in cured meat and fish products at low levels (for reviews, see 19, 42).

It has been shown that lactobacilli, group D streptococci, clostridia, and other bacteria will nitrosate secondary amines with nitrite at neutral pH values (48). The fact that nitrosation occurred at near neutral pH values was taken to indicate that the process was enzymatic, although no cell-free enzyme was obtained (49). Several species of streptococci including *S. faecalis, S. faecium,* and *S. lactis* have been shown to be capable of forming nitrosamines, but the other lactic acid bacteria and pseudomonads tested did not (18). These investigators found no evidence for an enzymatic reaction. *S. aureus* and halobacteria obtained from Chinese salted marine fish (previously shown to contain nitrosamines) produced nitrosamines when inoculated into salted fish homogenates containing 40 ppm of nitrate and 5 ppm nitrite (34).

Nitrite-sorbate and other nitrite combinations

In an effort to reduce the potential hazard of N-nitrosamine formation in bacon, the USDA in 1978 reduced the input NO_2 level for bacon to 120 ppm and set a 10 ppb maximum level for nitrosamines. While 120 ppm nitrite along with 550 ppm sodium ascorbate or sodium erythrobate is adequate to reduce the botulism hazard, it is desirable to reduce nitrite levels even further if protection against botulinal toxin production can be achieved. To this end, a proposal to allow the use of 40 ppm nitrite in combination with 0.26% potassium sorbate for bacon was made in 1978 but rescinded a year later when taste panel studies revealed undesirable effects. Meanwhile, many groups of researchers have shown that 0.26% sorbate in combination with 40 or 80 ppm nitrite is effective in preventing botulinal toxin production. Extensive reviews of these studies have been provided to which the reader is referred for more detailed information (77, 114, 123).

In an early study of the efficacy of 40 ppm nitrite + sorbate to prevent or delay botulinal toxin production in commercial-type bacon, Ivey et al. (57) used an inoculum of 1,100 types A and B spores/g and incubated the product at 27°C for up to 110 days. Time for the appearance of toxic samples when neither nitrite nor sorbate was used was 19 days. With 40 ppm nitrite and no sorbate, toxic samples appeared in 27 days, and for samples containing 40 ppm nitrite + 0.26% sorbate or no nitrite and 0.26% sorbate, > 110

days were required for toxic samples. This reduced nitrite level resulted in lower levels of nitrosopyrrolidine in cooked bacon. Somewhat different findings were reported by Sofos et al. (Table 11-2), with 80 ppm nitrite being required for the absence of toxigenic samples after 60 days. In addition to its inhibitory effects on *C. botulinum,* sorbate slows the depletion of nitrite during storage (113).

The effect of isoascorbate is to enhance nitrite inhibition by sequestering iron, although under some conditions it may reduce nitrite efficiency by causing a more rapid depletion of residual nitrite (124, 126). EDTA at 500 ppm appears to be even more effective than erythrobate in potentiating the nitrite effect, but only limited studies have been reported. Another chelate, 8-hydroxyquinoline, has been evaluated as a nitrite-sparing agent. When 200 ppm were combined with 40 ppm nitrite, a *C. botulinum* spore mixture of types A and B strains was inhibited for 60 days at 27°C in comminuted pork (92).

In an evaluation of the interaction of nitrite and sorbate, the relative effectiveness of the combination has been shown to be dependent upon other cure ingredients and product parameters. Employing a liver-veal agar medium at pH 5.8–6.0, the germination rate of *C. botulinum* type E spores decreased to nearly zero with 1.0, 1.5, or 2.0% sorbate; but with the same concentrations at pH 7.0–7.2, germination and outgrowth of abnormally shaped cells occurred (108). When 500 ppm nitrite was added to the higher-pH medium along with sorbate, cell lysis was enhanced. These investigators also found that 500 ppm linoleic acid alone at the higher pH prevented emergence and elongation of spores. Potassium sorbate significantly decreased toxin production by types A and B spores in pork slurries when NaCl was increased or pH and storage temperature were reduced (102). For chicken frankfurters, a sorbate-betalains mixture was found to be as effective as a conventional nitrite system for inhibiting *C. perfringens* growth (128).

Mode of action

It appears that nitrite inhibits *C. botulinum* by interfering with iron-sulfur enzymes such as ferrodoxin and thus preventing the synthesis of ATP from

Table 11-2. Effect of nitrite and sorbate on toxin production in bacon inoculated with *C. botulinum* types A and B spores and held up to 60 days at 27°C (114).

Treatment	*Percent Toxigenic*
Control (no NO_2, no sorbate)	90.0
0.26% sorbate, no $NaNO_2$	58.8
0.26% sorbate + 40 ppm $NaNO_2$	22.0
0.26% sorbate + 80 ppm $NaNO_2$	0.0
No sorbate, 120 ppm $NaNO_2$	0.4

pyruvate. The first direct finding in this regard was that of Woods et al. (132), who showed that the phosphoroclastic system of *C. sporogenes* is inhibited by nitric oxide, and later that the same occurs in *C. botulinum,* resulting in the accumulation of pyruvic acid in the medium (131).

The phosphoroclastic reaction involves the breakdown of pyruvate with inorganic phosphate and coenzyme A to yield acetyl phosphate. In the presence of ADP, ATP is synthesized from acetyl phosphate with acetate as the other product. In the breakdown of pyruvate, electrons are transferred first to ferredoxin, and from ferredoxin to H^+ to form H_2 in a reaction catalyzed by hydrogenase. Ferrodoxin and hydrogenase are iron-sulfur (nonheme) proteins or enzymes (11).

Following the work of Woods and Wood (131), the next most significant finding was that of Reddy et al. (94), who subjected extracts of nitrite-ascorbate-treated *C. botulinum* to electron spin resonance and found that nitric oxide reacted with iron-sulfur complexes to form iron-nitrosyl complexes. The presence of the latter results in the destruction of iron-sulfur enzymes such as ferrodoxin.

The resistance of the lactic acid bacteria to nitrite inhibition is well known, but the basis is just now clear: these organisms lack ferrodoxin. The clostridia contain both ferrodoxin and hydrogenase, which function in electron transport in the anaerobic breakdown of pyruvate to yield ATP, H_2, and CO_2. The ferrodoxin in clostridia has a molecular weight of 6,000 and contains 8 Fe atoms/mole and 8-labile sulfide atoms/mole.

Although the first definitive experimental finding was reported in 1981 as noted above, earlier work pointed to iron-sulfur enzymes as the probable nitrite targets. Among the first were O'Leary and Solberg (85), who showed that a 91% decrease occurred in the concentration of free —SH groups of soluble cellular compounds of *C. perfringens* inhibited by nitrite. Two years later, Tompkin et al. (125) offered the hypothesis that nitric oxide reacted with iron in the vegetative cells of *C. botulinum,* perhaps the iron in ferrodoxin. The inhibition by nitrite of active transport and electron transport was noted by several investigators, and these effects are consistent with nitrite inhibition of nonheme enzymes such as ferrodoxin and hydrogenase (105, 133). The enhancement of inhibition in the presence of sequestering agents may be due to the reaction of sequestrants to substrate iron: more nitrite becomes available for nitric oxide production and reaction with microorganisms.

Summary of nitrite effects

The following summary of the overall role and effects of nitrite in cured meats emphasizes the antibotulinal activities.

When added to processed meats such as wieners, bacon, smoked fish, and canned cured meats followed by substerilizing heat treatments, nitrite has definite antibotulinal effects. It also forms desirable product color and enhances flavor in cured meat products. The antibotulinal effect consists of inhibition of vegetative cell growth and the prevention of germination

and growth of spores that survive heat processing or smoking during post-processing storage. Clostridia other than *C. botulinum* are affected in a similar manner. While low initial levels of nitrite are adequate for color and flavor development, considerably higher levels are necessary for the antimicrobial effects.

When nitrite is heated in certain laboratory media, an antibotulinal factor or inhibitor is formed, the exact identity of which is not yet known. The inhibitory factor is the Perigo effect/factor or Perigo inhibitor. It does not form in filter-sterilized media. It develops in canned meats only when nitrite is present during heating. The initial level of nitrite is more important to antibotulinal activity than the residual level. Once formed, the Perigo factor is not affected greatly by pH changes.

Measurable preheating levels of nitrite decrease considerably during heating in meats and during postprocessing storage, more at higher storage temperatures than at lower.

The antibotulinal activity of nitrite is interdependent with pH, salt content, temperature of incubation, and numbers of botulinal spores. Heat-injured spores are more susceptible to inhibition than uninjured. Nitrite is more effective under Eh− than under Eh+ conditions.

Nitrite does not decrease the heat resistance of spores. It is not affected by ascorbate in its antibotulinal actions but does act synergistically with ascorbate in pigment formation.

Lactic acid bacteria are relatively resistant to nitrite (see above).

Endospores remain viable in the presence of the antibotulinal effect and will germinate when transferred to nitrite-free media.

Nitrite has a pK of 3.29 and consequently exists as undissociated nitrous acid at low pH values. The maximum undissociated state and consequent greatest antibacterial activity of nitrous acid are between pH 4.5 and 5.5.

With respect to its depletion or disappearance in ham, Nordin (84) found the rate to be proportional to its concentration and to be exponentially related to both temperature and pH. The depletion rate doubled for every 12.2°C increase in temperature or 0.86 pH unit decrease, and was not affected by heat denaturation of the ham. These relationships did not apply at room temperature unless the product was first heat treated, suggesting that viable organisms aided in its depletion.

It appears that the antibotulinal activity of nitrite is due to its inhibition of nonheme, iron-sulfur enzymes.

NaCl AND SUGARS

These compounds are grouped together because of the similarity in their modes of action in preserving foods. NaCl has been employed as a food preservative since ancient times (see Chapter 1). The early food uses of salt were for the purpose of preserving meats. This use is based upon the fact that at high concentrations, salt exerts a drying effect upon both food

and microorganisms. Nonmarine microorganisms may be thought of as normally possessing a degree of intracellular tonicity equivalent to that produced by about 0.85–0.9% NaCl. When microbial cells are suspended in salt (saline) of this concentration, the suspending menstrum can be said to be **isotonic** with respect to the cells. Since the amounts of NaCl and water are equal on both sides of the cell membrane, water moves across the cell membranes equally in both directions. When microbial cells are suspended in, say, a 5% saline solution, the concentration of water is greater inside the cells than outside (concentration of H_2O is highest where solute concentration is lowest). It should be recalled that in diffusion, water moves from its area of high concentration to its area of low concentration. In this case, water would pass out of the cells at a greater rate than it would enter. The result to the cell is **plasmolysis,** which results in growth inhibition and possibly death. This is essentially what is achieved when high concentrations of salt are added to fresh meats for the purpose of preservation. Both the microbial cells and those of the meat undergo plasmolysis (shrinkage), resulting in the drying of the meat as well as inhibition or death of microbial cells. To be effective, one must use enough salt to effect **hypertonic** conditions. The higher the concentration, the greater the preservative and drying effects. In the absence of refrigeration, fish and other meats may be effectively preserved by salting. The inhibitory effects of salt are not dependent upon pH as are some other chemical preservatives. Most nonmarine bacteria can be inhibited by 20% or less of NaCl, while some molds generally tolerate higher levels. Organisms that can grow in the presence of and require high concentrations of salt are referred to as **halophiles,** while those that can withstand but not grow in high concentrations are referred to as **halodurics.** The interaction of salt with nitrite and other agents in the inhibition of *C. botulinum* is discussed above under nitrites.

Sugars, such as sucrose, exert their preserving effect in essentially the same manner as salt. One of the main differences is in relative concentrations. It generally requires about six times more sucrose than NaCl to effect the same degree of inhibition. The most common uses of sugars as preserving agents are in the making of fruit preserves, candies, condensed milk, and the like. The shelf-stability of certain pies, cakes, and other such products is due in large part to the preserving effect of high concentrations of sugar, which, like salt, makes water unavailable to microorganisms.

Microorganisms differ in their response to hypertonic concentrations of sugars, with yeasts and molds being less susceptible than bacteria. Some yeasts and molds can grow in the presence of as much as 60% sucrose while most bacteria are inhibited by much lower levels. Organisms that are able to grow in high concentrations of sugars are designated **osmophiles,** while **osmoduric** microorganisms are those that are unable to grow but are able to withstand high levels of sugars. Some osmophilic yeasts such as *Saccharomyces rouxii* can grow in the presence of extremely high concentrations of sugars.

INDIRECT ANTIMICROBIALS

The compounds and products in this section are added to foods primarily for effects other than antimicrobial and are thus multifunctional food additives.

Antioxidants

Although used in foods primarily to prevent the auto-oxidation of lipids, the phenolic antioxidants listed in Table 11-3 have been shown to possess antimicrobial activity against a wide range of microorganisms including some viruses, mycoplasmas, and protozoa. These compounds have been evaluated extensively as nitrite-sparing agents in processed meats and in combination with other inhibitors, and several excellent reviews have been made (10, 37, 65).

Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and *t*-butylhydroxyquinoline (TBHQ) are inhibitory to gram-positive and gram-negative bacteria as well as to yeasts and molds at concentrations ranging from about 10 to 1,000 ppm depending upon substrate. In general, higher concentrations are required to inhibit in foods than in culture media, especially in high-fat foods. BHA was about fifty times less effective against *Bacillus* spp. in strained chicken than in nutrient broth (110). BHA, BHT, TBHQ, and propyl gallate (PG) were all less effective in ground pork than in culture media (38). While strains of the same bacterial species may show wide variation in sensitivity to either of these antioxidants, it appears that BHA

Table 11-3. Some GRAS indirectly antimicrobial chemicals used in foods.

Compound	*Primary use*	*Most susceptible organisms*
Butylated hydroxyanisole (BHA)	Antioxidant	Bacteria, some fungi
Butylated hydroxytoluene (BHT)	Antioxidant	Bacteria, viruses, fungi
t-butylhydroxyquinoline (TBHQ)	Antioxidant	Bacteria, fungi
Propyl gallate (PG)	Antioxidant	Bacteria
Nordihydroguaiaretic acid	Antioxident	Bacteria
Ethylenediamine tetraacetic acid (EDTA)	Sequestrant/ stabilizer	Bacteria
Sodium citrate	Buffer/sequestrant	Bacteria
Lauric acid	Defoaming agent	Gram-positive bacteria
Monolaurin	Emulsifier	Gram-positive bacteria, yeasts
Diacetyl	Flavoring	Gram-negative bacteria, fungi
d- and *l*-carvone	Flavoring	Fungi, gram-positive bacteria
Phenylacetaldehyde	Flavoring	Fungi, gram-positive bacteria
Menthol	Flavoring	Bacteria, fungi
Vanillin, ethyl vanillin	Flavoring	Fungi
Spices/spice oils	Flavoring	Bacteria, fungi

and TBHQ are more inhibitory than BHT to bacteria and fungi, while the latter is more viristatic. To prevent growth of *C. botulinum* in a prereduced medium, 50 ppm BHA and 200 ppm BHT were required, while 200 ppm PG were ineffective (99). Employing 16 gram-negative and 8 gram-positive bacteria in culture media, Gailani and Fung (38) found the gram positives to be more susceptible than gram negatives to BHA, BHT, TBHQ, and PG with each being more effective in nutrient agar than in BHT broth. In nutrient agar the relative effectiveness was BHA > PG > TBHQ > BHT, while in BHI, TBHQ > PG > BHA > BHT.

Food-borne pathogens such as *B. cereus, V. parahaemolyticus,* salmonellae, and *S. aureus* are effectively inhibited at concentrations < 500 ppm, while some are sensitive to as little as 10 ppm. The pseudomonads, especially *P. aeruginosa,* are among the most resistant bacteria. Three toxin-producing penicillia were inhibited significantly in salami by BHA, TBHQ, and a combination of these two at 100 ppm, while BHT and PG were ineffective (74). Combinations of BHA/sorbate and BHT/monolaurin have been shown to be synergistic against *S. aureus* (10, 21), and BHA/sorbate against *S. typhimurium* (21). BHT/TBHQ has been shown to be synergistic against aflatoxin-producing penicillia (74).

Flavoring agents

Of the many agents used to impart aromas and flavors to foods, some possess definite antimicrobial effects. In general, flavor compounds tend to be more antifungal than antibacterial. The nonlactic, gram-positive bacteria are the most sensitive and the lactic acid bacteria are rather resistant. The essential oils and spices have received the most attention by food microbiologists, while the aroma compounds have been studied more for their use in cosmetics and soaps.

Of twenty-one flavoring compounds examined in one study, about one-half had minimal inhibitory concentrations (MIC) of 1,000 ppm or less against either bacteria or fungi (61). All were pH sensitive, with inhibition increasing as pH and temperature of incubation decreased. Some of these compounds are noted in Table 11-3.

One of the most effective flavoring agents is diacetyl, which imparts the aroma of butter (58). It is somewhat unique in being more effective against gram-negative bacteria and fungi than against gram-positive bacteria. In plate count agar at pH 6.0 and incubation at 30°C, all but one of twenty-five gram-negative bacteria and fifteen of sixteen yeasts and molds were inhibited by 300 ppm (59). At pH 6.0 and incubation at 5°C in nutrient broth, < 10 ppm inhibited *P. fluorescens, P. geniculata,* and *S. faecalis,* while under the same conditions except with incubation at 30°C, about 240 ppm were required to inhibit these and other organisms (61). It appears that diacetyl antagonizes arginine utilization by reacting with arginine-binding proteins of gram-negative bacteria. The greater resistance of gram-positive bacteria appears to be due to their lack of similar periplasmic binding

proteins and their possession of larger amino acid pools. Another flavor compound that imparts the aroma of butter is 2,3-pentanedione, and it has been found to be inhibitory to a limited number of gram-positive bacteria and fungi at 500 ppm or less (61).

The agent *l*-carvone imparts spearmintlike and the agent *d*-carvone imparts carawaylike aromas, and both are antimicrobial with the *l*-isomer being more effective than the *d*-isomer, while both are more effective against fungi than bacteria at 1,000 ppm or less (61). Phenylacetaldehyde imparts a hyacinthlike aroma, and has been shown to be inhibitory to *S. aureus* at 100 ppm and *Candida albicans* at 500 ppm (61, 83). Menthol, which imparts a peppermintlike aroma, was found to inhibit *S. aureus* at 32 ppm, and *E. coli* and *C. albicans* at 500 ppm (61, 83). Vanillin and ethyl vanillin are inhibitory, especially to fungi at levels < 1,000 ppm.

Spices and essential oils

While used primarily as flavoring and seasoning agents in foods, many spices possess significant antimicrobial activity. In all instances, antimicrobial activity is due to specific chemicals or essential oils, some of which are noted in Chapter 3. The search for nitrite-sparing agents generated new interest in spices and spice extracts in the late 1970s (much of this work has been reviewed by Shelef, 109).

It would be difficult to predict what antimicrobial effects if any are derived from spices as they are used in foods, for the quantities employed differ widely depending upon taste and the relative effectiveness varies depending upon product composition. Because of the varying concentrations of the antimicrobial constituents in different spices, and because many studies have been conducted employing them on a dry weight basis, it is difficult to ascertain the MIC of given spices against specific organisms. Another reason for conflicting results by different investigators is the assay method employed. In general, higher MIC values are obtained when highly volatile compounds are evaluated on the surface of plating media than when they are tested in pour plates or broth. When eugenol was evaluated by surface plating onto PCA at pH 6, only nine of fourteen gram-negative and twelve of twenty gram-positive bacteria (including eight lactics) were inhibited by 493 ppm, while in nutrient broth at the same pH, MICs of 32 and 63 were obtained for *Torulopsis candida* and *Aspergillus niger,* and *S. aureus* and *E. coli,* respectively (61). It has been noted that spice extracts are less inhibitory in media than spices, and this is probably due to a slower release of volatiles by the latter (111). In spite of the difficulties of comparing results from study to study, the antimicrobial activity of spices is unquestioned and a large number of investigators have shown the effectiveness of at least twenty different spices or their extracts against most food-poisoning organisms including mycotoxigenic fungi (109).

In general, spices are less effective in foods than in culture media, and gram-positive bacteria are more sensitive than gram negatives, with the

lactic acid bacteria being the most resistant among gram positives (134). While results concerning them are debatable, the fungi appear to be in general more sensitive than gram-negative bacteria. Some gram negatives, however, are highly sensitive. Antimicrobial substances vary in content from the allicin of garlic (with a range of 0.3–0.5%) to eugenol in cloves (16–18%) (109). When whole spices are employed, MIC values range from 1–5% for sensitive organisms. Sage and rosemary are among the most antimicrobial as reported by various researchers, and it has been reported that 0.3% in culture media inhibited twenty-one of twenty-four gram-positive bacteria and were more effective than allspice (111).

With respect to specific inhibitory levels of extracts and essential oils, Huhtanen (53) made ethanol extracts of thirty-three spices, tested them in broth against *C. botulinum*, and found that achiote and mace extracts produced an MIC of 31 ppm and were the most effective of the thirty-three. Next most effective were nutmeg, bay leaf, and white and black peppers with MICs of 125 ppm. Employing the essential oils of oregano, thyme, and sassafras, Beuchat (4) found that 100 ppm were cidal to *V. parahaemolyticus* in broth. Growth and aflatoxin production by *A. parasiticus* in broth were inhibited by 200–300 ppm of cinnamon and clove oils, by 150 ppm cinnamic aldehyde, and by 125 ppm eugenol (12).

The mechanisms by which spices inhibit microorganisms are unclear and may be presumed to be different for unrelated groups of spices. That the mechanism for oregano, rosemary, sage, and thyme may be similar is suggested by the finding that resistance development by some lactic acid bacteria to one was accompanied by resistance to the other three (134).

Medium-chain fatty acids and esters

Acetic, propionic, and sorbic acids are short-chain fatty acids used primarily as preservatives. Medium-chain fatty acids, however, are employed primarily as surface-active or emulsifying agents. The antimicrobial activity of the medium-chain fatty acids is best known from soaps, which are salts of fatty acids. Those most commonly employed are composed of twelve to sixteen carbons. For saturated fatty acids, the most antimicrobial chain length is C_{12}; for monounsaturated (containing 1 double bond) $C_{16:1}$; and for polyunsaturated (containing more than one double bond) $C_{18:2}$ is the most antimicrobial (66). In general, fatty acids are effective primarily against gram-positive bacteria and yeasts. While the C_{12} to C_{16} chain lengths are the most active against bacteria, the C_{10} to C_{12} are most active against yeasts (66). Fatty acids and esters and the structure-function relationships among them have been reviewed and discussed by Kabara (65, 66). Saturated aliphatic acids effective against *C. botulinum* have been evaluated by Dymicky and Trenchard (32).

The monoesters of glycerol and the diesters of sucrose have been found to be more antimicrobial than the corresponding free fatty acids, and to compare favorably with sorbic acid and the parabens as antimicrobials (65).

Monolaurin is the most effective of the glycerol monoesters, while sucrose dicaprylate is the most effective of the sucrose diesters. Monolaurin (lauricidin) has been evaluated by a large number of investigators and found to be inhibitory to a variety of gram-positive bacteria and some yeasts at 5–100 ppm (10, 65). Unlike the short-chain fatty acids, which are most effective at low pH, monolaurin is effective over the range 5.0 to 8.0 (67).

Because the fatty acids and esters have a narrow range of effectiveness and GRAS substances such as EDTA, citrate, and phenolic antioxidants also have limitations as antimicrobial agents when used alone, Karara (65, 66) has stressed the "preservative system" approach to the control of microorganisms in foods by using combinations of chemicals to fit given food systems and preservation needs. By this approach, a preservative system might consist of three compounds—monolaurin/EDTA/BHA, for example. While EDTA possesses little antimicrobial activity by itself, it renders gram-negative bacteria more susceptible by rupturing the outer membrane and thus potentiating the effect of fatty acids or fatty acid esters. An antioxidant such as BHA would exert effects against bacteria and molds and serve as an antioxidant at the same time. By use of such a system, the development of resistant strains could be minimized and the pH of a food could become less important relative to the effectiveness of the inhibitory system.

ACETIC AND LACTIC ACIDS

These two organic acids are among the most widely employed as preservatives. In most instances, their origin to the subject foods is due to their production within the food by lactic acid bacteria. Products such as pickles, sauerkraut, and fermented milks, among others, are created by the fermentative activities by various lactic acid bacteria, which produce acetic, lactic, and other acids (see Chapter 16 for fermented foods, and the review by Doores, 28, for further information).

The antimicrobial effect of organic acids such as propionic and lactic is due both to the depression of pH below the growth range and metabolic inhibition by the undissociated acid molecules. In determining the quantity of organic acids in foods, titratable acidity is of more value than pH alone, since the latter is a measure of hydrogen ion concentration and organic acids do not ionize completely. In measuring titratable acidity, the amount of acid that is capable of reacting with a known amount of base is determined. The titratable acidity of products such as sauerkraut is a better indicator of the amount of acidity present than pH.

ANTIBIOTICS

While no antibiotic is legally permissible as a food additive in the United States at the present time, two are approved for food use in many other

countries (nisin and natamycin), and three others (tetracyclines, subtilin, and tylosin) have been studied and found effective for various food applications. The early history, efficacy, and applications of most were reviewed in 1966 (78), and all have been reviewed and discussed more recently (60). Detailed reviews on nisin have been provided by Hurst (54, 55) and Lipinska (75).

Three antibiotics have been investigated extensively as heat adjuncts for canned foods (subtilin, tylosin, and nisin). Nisin, however, is used most widely in cheeses. Chlortetracycline and oxytetracycline were widely studied for their application to fresh foods while natamycin is employed as a food fungistat.

While in general the use of chemical preservatives in foods is not popular among many consumers, the idea of employing antibiotics is even less popular. Some risks may be anticipated from the use of any food additive, but the risks should not outweigh the benefits overall. The general view in the United States at the present time is that the benefits to be gained by using antibiotics in foods do not outweigh the risks, some of which are known and some of which are presumed. Some fifteen considerations on the use of antibiotics as food preservatives were noted by Ingram et al. (56), and several of the key ones are summarized below:

1. The antibiotic agent should kill, not inhibit the flora, and should ideally decompose into innocuous products, or be destroyed on cooking for products that require cooking.
2. The antibiotic should not be inactivated by food components or products of microbial metabolism.
3. The antibiotic should not readily stimulate the appearance of resistant strains.
4. The antibiotic should not be used in foods if used therapeutically or as an animal feed additive.

It may be noted from the summary comparison in Table 11-4 that the tetracyclines are used both clinically and as feed additives while tylosin is used in animal feeds and only in the treatment of some poultry diseases. Neither nisin nor subtilin is used medically or in animal feeds, and while nisin is used in many countries, subtilin is not. The structural similarities of these two antibiotics may be noted from Fig. 11-2.

Nisin

This is a polypeptide antibiotic structurally related to subtilin, but unlike subtilin it does not contain tryptophane residues (Fig. 11-2). While the C-terminal amino acids are similar, the N-terminals are not. The first food use of nisin was by Hirsch et al. (51) to prevent the spoilage of Swiss cheese by *Clostridium butyricum*. It is clearly the most widely used antibiotic for food preservation, with around thirty-nine countries permitting its use

in foods to varying degrees (for a list of countries, see Hurst, 54). It is not permitted in foods in the United States and Canada. Among some of its desirable properties as a food preservative are the following: (1) it is nontoxic, (2) it is produced naturally by *Streptococcus lactis* strains, (3) it is heat stable and has excellent storage stability, (4) it is destroyed by digestive enzymes, (5) it does not contribute to off-flavors or off-odors, and (6) it has a narrow spectrum of antimicrobial activity. The compound is effective against gram-positive bacteria, primarily sporeformers, and ineffective against fungi and gram-negative bacteria. *Enterococcus* (*Streptococcus*) *faecalis* is one of the most resistant gram positives.

A large amount of research has been carried out with nisin as a heat-adjunct in canned foods, or as an inhibitor of heat-shocked spores of *Bacillus*

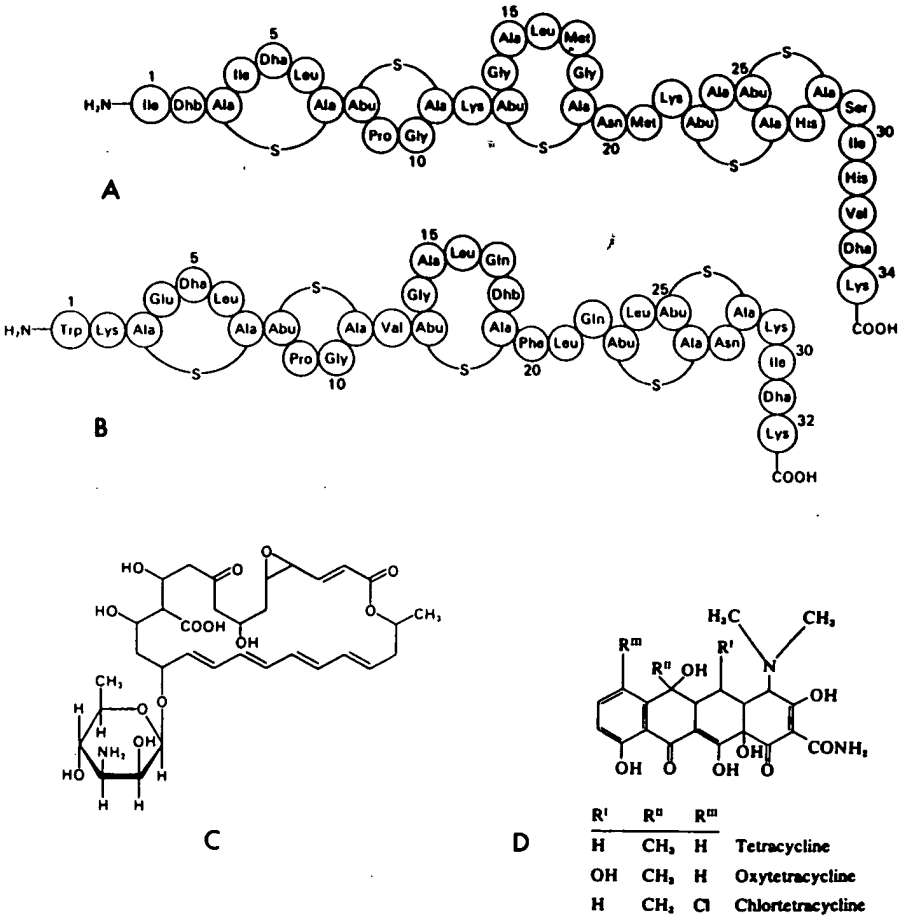


Fig. 11-2. Structural formulae of nisin (A), subtilin (B), natamycin (C), and the tetracyclines (D).

and *Clostridium* strains, and the MIC for preventing outgrowth of germinating spores ranges widely from 3 to > 5,000 IU/ml or < 1 to > 125 ppm (1 μg of pure nisin is about 40 IU or RU—Reading unit) (54). Depending upon the country and the particular food product, typical usable levels are in the range of about 2.5 to 100 ppm, although some countries do not impose concentration limits.

A conventional heat process for low-acid canned foods requires an F_o treatment of 6–8 (see Chapter 14) to inactivate the endospores of both *C. botulinum* and spoilage organisms. By adding nisin the heat process can be reduced to an F_o of 3 (to inactivate *C. botulinum* spores) resulting in increased product quality of low-acid canned foods. While the low-heat treatment will not destroy the endospores of spoilage organisms, nisin prevents their germination by acting early in the endospore germination cycle (Fig. 11-1). In addition to its use in certain canned foods, nisin is most often employed in dairy products—processed cheeses, condensed milk, pasteurized milk, and so on. Some countries permit its use in processed tomato products and canned fruits and vegetables (54). It is most stable in acidic foods.

Because of the effectiveness of nisin in preventing the outgrowth of germinating endospores of *C. botulinum* and the search to find safe substances that might replace nitrites in processed meats, this antibiotic has been studied as a possible replacement for nitrite. While some studies showed encouraging results employing *C. sporogenes* and other nonpathogenic organisms, a recent study employing *C. botulinum* types A and B spores in pork slurries indicated the inability of nisin at concentrations up to 550 ppm in combination with 60 ppm nitrite to inhibit spore outgrowth (93). Employed in culture media without added nitrite, the quantity of nisin required for 50% inhibition of *C. botulinum* type E spores was 1–2 ppm; 10–20 ppm for type B; and 20–40 ppm for type A (107). The latter authors found that higher levels were required for inhibition in cooked meat medium than in TPYG medium and suggested that nisin was approximately equivalent to nitrite in preventing the outgrowth of *C. botulinum* spores.

With respect to mode of action, nisin and subtilin may be presumed to act similarly since they are both polypeptide antibiotics with highly similar structures. They act at the same site on germinating endospores (Fig. 11-1). Some of the polypeptide antibiotics typically attack cell membranes and act possibly as surfactants or emulsifying agents on membrane lipids. These agents may be presumed to inhibit gram-positive bacteria by inhibiting cell wall murein synthesis since bacitracin (another polypeptide antibiotic that also inhibits gram-positive bacteria) is known to inhibit murein synthesis (47). That nisin affects murein synthesis has been shown by Reisinger et al. (95), and this finding is not inconsistent with its lack of toxicity for man. A similar lack of toxicity for subtilin may be presumed.

Natamycin

This antibiotic (also known as pimaricin, tennecetin, and myprozine) is a polyene that is quite effective against yeasts and molds but not bacteria.

Natamycin is the international nonproprietary name since it was isolated from *Streptomyces natalensis*. Its structural formula is presented in Fig. 11-2.

In granting the acceptance of natamycin as a food preservative, the joint FAO/WHO Expert Committee (35) took the following into consideration: (1) it does not affect bacteria, (2) it stimulates an unusually low level of resistance among fungi, (3) it is rarely involved in cross-resistance among other antifungal polyenes, and (4) DNA transfer between fungi does not occur to the extent that it does with some bacteria. Also, from Table 11-4 it may be noted that its use is limited as a clinical agent, and it is not used as a feed additive. Natamycin has been shown by numerous investigators to be very effective against both yeasts and molds, and many of these reports have been summarized (60).

The relative effectiveness of natamycin was compared to sorbic acid and four other antifungal antibiotics by Klis et al. (69) for the inhibition of sixteen different fungi (mostly molds), and while from 100 to 1,000 ppm sorbic acid were required for inhibition, from 1 to 25 ppm natamycin were effective against the same strains in the same media. To control fungi on strawberries and raspberries, natamycin was compared with rimocidin and nystatin, and it along with rimocidin was effective at levels of 10–20 ppm, while 50 ppm nystatin were required for effectiveness (1). In controlling fungi on salami, the spraying of fresh salami with a 0.25% solution was found to be effective by one group of investigators (50), but another researcher was unsuccessful in his attempts to prevent surface-mold growth on Italian dry sausages when they were dipped in a 2,000-ppm solution (53). Natamycin

Table 11-4. Summary comparison of some properties of the antibiotics discussed in this chapter (60).

Property	*Tetracyclines*	*Subtilin*	*Tylosin*	*Nisin*	*Natamycin*
Widely used in foods	No	No	No	Yes	Yes
First food use	1950	1950	1961	1951	1956
Chemical nature	Tetracycline	Poly-peptide	Macro-lide	Poly-peptide	Polyene
Used as heat adjunct	No	Yes	Yes	Yes	No
Heat stability	Sensitive	Stable	Stable	Stable	Stable
Microbial spectrum	G^+, G^-	G^+	G^+	G^+	Fungi
Used medically	Yes	No	Yes[a]	No	Yes[b]
Used in feeds	Yes	No	Yes	No	No

[a] In treating poultry diseases.
[b] Limited.

spray (2 × 1,000 ppm) was as good as or slightly better than 2.5% potassium sorbate.

Natamycin appears to act in the same manner as other polyene antibiotics—by binding to membrane sterols and inducing distortion of selective membrane permeability (45). Since bacteria do not possess membrane sterols, their lack of sensitivity to this agent is thus explained. Mycoplasmae, however, do have membrane sterols, but whether this antibiotic is effective against this group is unclear.

Tetracyclines

Chlortetracycline (CTC) and oxytetracycline (OTC) were approved by the FDA in 1955 and 1956, respectively, at a level of 7 ppm to control bacterial spoilage in uncooked refrigerated poultry, but these approvals were subsequently rescinded. The efficacy of this group of antibiotics in extending the shelf life of refrigerated foods was first established by Tarr and associates in Canada working with fish (120). Subsequent research by a large number of workers in many countries established the effectiveness of CTC and OTC in delaying bacterial spoilage of not only fish and seafoods but poultry, red meats, vegetables, raw milk, and other foods (for a review of food applications, see 60, 78). CTC is generally more effective than OTC. The surface treatment of refrigerated meats with 7–10 ppm typically results in shelf-life extensions of at least 3–5 days and a shift in ultimate spoilage flora from gram-negative bacteria to yeasts and molds. When CTC is combined with sorbate to delay spoilage of fish, the combination has been shown to be effective for up to 14 days. Rockfish fillets dipped in a solution of 5 ppm CTC and 1% sorbate had significantly lower APCs after vacuum-package storage at 2°C after 14 days than controls (81).

The tetracyclines are both heat sensitive and storage labile in foods, and these factors were important in their initial acceptance for food use. As may be noted from Table 11-4, they are used to treat diseases in man and animals and are used also in feed supplements. The risks associated with their use as food preservatives in developed countries seem clearly to outweigh the benefits.

Subtilin

This antibiotic was discovered and developed by scientists at the Western Regional Laboratory of the USDA, and its properties were described by Dimick et al. (27). As noted above, it is structurally similar to nisin (Fig. 11-2) even though it is produced by some strains of *B. subtilis*. Like nisin, it is effective against gram-positive bacteria, is stable to acid, and possesses enough heat resistance to withstand destruction at 121°C for 30 to 60 min. Subtilin is effective in canned foods at levels of 5 to 20 ppm in preventing the outgrowth of germinating endospores, and its site of action is the same as for nisin (Fig. 11-1). Like nisin, it is used neither in the treatment of

human or animal infections nor as a feed additive. This antibiotic may be just as effective as nisin even though it has received little attention since the late 1950s. Its mode of action is discussed above along with that of nisin, and its development and evaluation have been reviewed (60).

Tylosin

This antibiotic is a nonpolyene macrolide as are the clinically useful antibiotics erythromycin, oleandomycin, and others. It is more inhibitory than nisin or subtilin. Denny et al. (23) were apparently the first to study its possible use in canned foods. When 1 ppm was added to cream-style corn containing flat-sour spores and given a "botulinal" cook, no spoilage of product occurred after 30 days with incubation at 54°C (22). Similar findings were made by others in the 1960s, and these have been summarized (60).

Unlike nisin, subtilin, and natamycin, tylosin is used in animal feeds and also to treat some diseases of poultry. As a macrolide, it is most effective against gram-positive bacteria. It inhibits protein synthesis by associating with the 50S ribosomal subunit, and shows at least partial cross-resistance with erythromycin.

ANTIFUNGAL AGENTS FOR FRUITS

Listed in Table 11-5 are some compounds applied to fruits after harvest to control fungi, primarily molds. **Benomyl** is applied uniformly over the entire surface of fruits, examples of which are noted in the table. It is applied at concentrations of 0.5–1.0 g/liter. It can penetrate the surface of some vegetables, and is used world-wide to control crown rot and anthracnose of bananas, and stem-end rots of citrus fruits. It is more effective than **thi-**

Table 11-5. Some chemical agents employed to control fungal spoilage of fresh fruits (33).

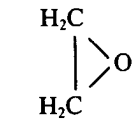
Compound	*Fruits*
Thiabendazole	Apples, pears, citrus fruits, pineapples
Benomyl	Apples, pears, bananas, citrus fruits, mangoes, papayas, peaches, cherries, pineapples
Biphenyl	Citrus fruits
SO_2 fumigation	Grapes
Sodium-α-phenylphenate	Apples, pears, citrus fruits, pineapples

bendazole and penetrates with greater ease. Both benomyl and thiabendazole are effective in controlling dry rot caused by *Fusarium* spp. To prevent the spread of *Botrytis* from grape to grape, SO_2 is employed for long-term storage. It is applied shortly after harvest and about once a week thereafter. A typical initial treatment consists of a 20-min application of a 1% preparation, and about 0.25% in subsequent treatments (the use of SO_2 in other foods is discussed above).

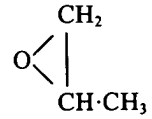
Biphenyl is used to control the decay of citrus fruits by penicillia for long-distance shipments and is generally impregnated into fruit wraps or sheets between fruit layers.

ETHYLENE AND PROPYLENE OXIDES

Ethylene and propylene oxides along with ethyl and methyl formate ($HCOOC_2H_5$ and $HCOOCH_3$, respectively) are treated together in this section because of their similar actions. The structures of the oxide compounds are as follows:



Ethylene oxide



Propylene oxide

The oxides exist as gases and are employed as fumigants in the food industry. The oxides are applied to dried fruits, nuts, spices, and so forth, primarily as antifungal compounds.

Ethylene oxide is an alkylating agent (91) and its antimicrobial activity is presumed to be related to this action in the following manner. In the presence of labile H atoms, the unstable three-membered ring of ethylene oxide splits. The H atom attaches itself to the oxygen, forming a hydroxyl ethyl radical, CH_2CH_2OH, which in turn attaches itself to the position in the organic molecule left vacant by the H atom. As a result, the hydroxyl ethyl group blocks reactive groups within microbial proteins, thus resulting in inhibition. Among the groups capable of supplying a labile H atom are the following: —COOH, —NH_2, —SH, and —OH. Ethylene oxide appears to affect endospores of *C. botulinum* by alkylation of guanine and adenine components of spore DNA (80, 130).

Ethylene oxide is used as a gaseous sterilant for flexible and semirigid containers for packaging aseptically processed foods. All of the gas dissipates from the containers following their removal from treatment chambers. With respect to its action on microorganisms, it is not much more effective against vegetative cells than it is against endospores, as can be seen from the D values given in Table 11-6.

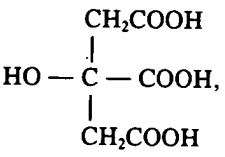
Table 11-6. D values for four chemical sterilants of some food-borne microorganisms.

Organism	*D*[a]	*Conc.*	*Temp.*[b]	*Condition*	*Reference*
		Hydrogen peroxide			
C. botulinum 169B	0.03	35%	88		121
B. coagulans	1.8	26%	25		122
B. stearothermophilus	1.5	26%	25		122
B. subtilis ATCC 95244	1.5	20%	25		116
B. subtilis A	7.3	26%	25		121
		Ethylene oxide			
C. botulinum 62A	11.5	700 mg/L	40	47% R.H.	106
C. botulinum 62A	7.4	700 mg/L	40	23% R.H.	130
C. sporogenes ATCC 7955	3.25	500 mg/L	54.4	40% R.H.	68
B. coagulans	7.0	700 mg/L	40	33% R.H.	7
B. coagulans	3.07	700 mg/L	60	33% R.H.	7
B. stearothermophilus ATCC 7953	2.63	500 mg/L	54.4	40% R.H.	68
L. brevis	5.88	700 mg/L	30	33% R.H.	7
M. radiodurans	3.00	500 mg/L	54.4	40% R.H.	68
		Sodium hypochlorite			
A. niger conidiospores	0.61	20 ppm[c]	20	pH 3.0	15
A. niger conidiospores	1.04	20 ppm[c]	20	pH 5.0	15
A. niger conidiospores	1.31	20 ppm[c]	20	pH 7.0	15
		Iodine ($\frac{1}{2}I_2$)			
A. niger conidiospores	0.86	20 ppm[c]	20	pH 3.0	15
A. niger conidiospores	1.15	20 ppm[c]	20	pH 5.0	15
A. niger conidiospores	2.04	20 ppm[c]	20	pH 7.0	15

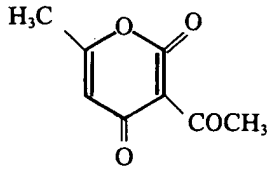
[a] In minutes;
[b] °C;
[c] As Cl.

MISCELLANEOUS CHEMICAL PRESERVATIVES

Sodium diacetate ($CH_3COONa \cdot CH_3COOH \cdot xH_2O$), a derivative of acetic acid, is used in bread and cakes to prevent moldiness. Organic acids such as **citric,**

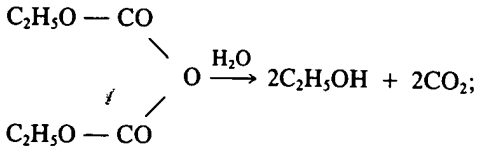


exert a preserving effect on foods such as soft drinks. **Hydrogen peroxide** (H_2O_2) has received limited use as a food preservative. In combination with heat, it has been used in milk pasteurization and sugar processing, but its widest use is as a sterilant for food-contact surfaces of olefin polymers and polyethylene in aseptic packaging systems (see Chapter 14). The D values of some food-borne microorganisms are presented in Table 11-6. **Ethanol** (C_2H_5OH) is present in flavoring extracts and effects preservation by virtue of its desiccant and denaturant properties. **Dehydroacetic acid,**

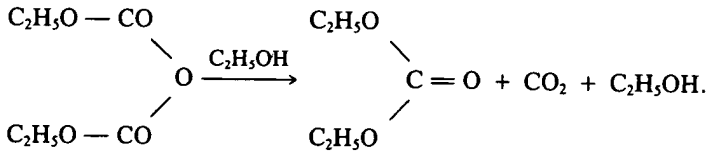


is used to preserve squash. **Diethylpyrocarbonate** has been used in bottled wines and soft drinks as a yeast inhibitor. It decomposes to form ethanol and CO_2 by either hydrolysis or alcoholysis (39):

Hydrolysis (reaction with water):



Alcoholysis (reaction with ethyl alcohol):



Saccharomyces cerevisiae and conidia of *Aspergillus niger* and *Byssochlamys fulva* have been shown to be destroyed by this compound during the first ½ h of exposure, while the ascospores of *B. fulvä* required 4 to 6 h for maximal destruction (115). Cidal concentrations for yeasts range from about 20 to 1,000 ppm depending upon species or strain. *Lactobacillus plantarum* and *Leuconostoc mesenteroides* required 24 h or longer for destruction. Sporeforming bacteria are quite resistant to this compound. Sometimes urethane is formed when this compound is used and because it is a carcinogen, the use of diethylpyrocarbonate is no longer permissible in the United States.

Wood smoke imparts certain chemicals to smoked products that enable these products to resist microbial spoilage. One of the most important is formaldehyde (CH_2O), which has been known for many years to possess antimicrobial properties. This compound acts as a protein denaturant by virtue of its reaction with amino groups. Also in wood smoke are aliphatic acids, alcohols, ketones, phenols, higher aldehydes, tar, methanol, cresols, and other compounds (30), all of which may contribute to the antibacterial actions of meat smoking. Since a certain amount of heat is necessary to produce smoke, part of the shelf-stability of smoked products is due to heat destruction of surface organisms as well as to the drying that occurs. A study of the antibacterial activity of liquid smoke by Handford and Gibbs (46) revealed that little activity occurred at concentrations of smoke that produced acceptable smoked flavor. Employing an agar medium containing 1:1 dilution of smoked water, these investigators found that micrococci and staphylococci were slightly more inhibited than the lactic acid bacteria. The overall combined effect of smoking and vacuum packaging results in a reduction of numbers of catalase-positive bacteria on the smoked product, while the catalase-negative lactic acid bacteria are better able to withstand the low Eh conditions of vacuum-packaged products.

The **lactoperoxidase system** is an inhibitory system that occurs naturally in bovine milk. It consists of three components: lactoperoxidase, thiocyanate, and H_2O_2. All three components are required for antimicrobial effects, and the gram-negative psychrotrophs such as the pseudomonads are quite sensitive. The quantity of lactoperoxidase needed is 0.5–1.0 ppm, while bovine milk normally contains about 30 ppm (5). While both thiocyanate and H_2O_2 occur normally in milk, the quantities vary. For H_2O_2 about 100 U/ml are required in the inhibitory system, while only 1–2 U/ml normally occurs in milk. An effective level of thiocyanate is around 0.25 mM, while in milk the quantity varies between 0.02–0.25 mM (5).

When the lactoperoxidase system in raw milk was activated by adding thiocyanate to 0.25 mM along with an equimolar amount of H_2O_2, the shelf life was extended to 5 days compared to 48 h for controls (5). The system was more effective at 30 than at 4°C. The bactericidal effect increases with acidity, and the cytoplasmic membrane appears to be the cell target. In addition to the direct addition of H_2O_2, an exogenous source can be provided by the addition of glucose and glucose oxidase. To avoid the direct addition of glucose oxidase, this enzyme has been immobilized on glass beads so that glucose is generated only in the amounts needed by the use of immobilized β-galactosidase (6). The lactoperoxidase system can be used to preserve raw milk in countries where refrigeration is uncommon. The addition of about 12 ppm of SCN^- and 8 ppm of H_2O_2 should be harmless to the consumer (97). The system has been described in detail by Law and Reiter (72), Law and Mabbitt (71), and Reiter (96); and reviewed by Reiter and Harnulv (97).

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